Supporting Information:

The conformation of P450cam in complex with putidaredoxin is dependent on oxidation state

William K. Myers, Young-Tae Lee, R. David Britt, and David B. Goodin

Methods

Sample preparation.

Protein purification and spin-labeling with MTSL were carried out as described previously [14] . All proteins were initially exchanged into 71.4 mM Tris (pH 7.4), 214 mM KCl and 1.4 mM camphor in 99% D_2O. After adding 30% d_8-glycerol, the final EPR samples have 50 mM Tris (pH 7.4), 150 mM KCl, 1 mM camphor in 70% D_2O/30% d_8-glycerol. Concentration of P450cam was fixed at 150 μM for all EPR samples. For samples for ferric P450cam and oxidized Pdx, both proteins were mixed together before adding d_8-glycerol. For CO-bound reduced samples, proteins were reduced biologically to prevent reduction of MTSL spin label by dithionite. P450cam (150 μM), Pdx (3 – 150 μM) and Pdr (3 μM) were placed in a gas-tight vial A (80 μL). 1 mM NADH and d_8-glycerol were placed in a gas-tight vial B (120 μL). Then CO was blown extensively into both vials. After mixing proteins and NADH from each vials in the glove box, samples were flash-frozen in liquid nitrogen also inside the glove box. Samples for UV-visible absorption spectra were prepared in protonated buffers and glycerol. Since the sample concentration was high (150 μM), a gas-tight 1mm cuvette was used to prevent any necessary dilution for UV-visible absorption spectroscopy.

EPR experiments

Data were acquired at the CalEPR center at UC Davis, CW-EPR on a Bruker E500 spectrometer with a Super X bridge, ESR-900 cryostat, and SHQE-W resonator, and DEER on a Bruker E580 spectrometer at 9.5 GHz modified for DEER. Bridge modifications include use of a HP 8761A SPDT switch to input the pump frequency from a HP 83622A source to the manual pulse forming unit (MPFU). A Bruker MS5 split-ring resonator was used in an Oxford Instruments CF935 cryostat, at a temperature of 30 or 50 K and a magnetic field of about 341 mT. The DEER pulse sequence $(\pi/2)_1 - \tau_1 - (\pi)_1 - \tau_1 + T - (\pi)_2 - (\tau_2 - T) - (\pi)_1 - \tau_2$ - [echo] was used with pulse lengths of 16/32/32 ns for the probe pulses (subscript 1) and 16 ns for the pump pulse (subscript 2). Pulse lengths were obtained by minimizing TWTA input power at the observing frequency, followed by minimizing the pump π-pulse width within the HP source power leveler range (15 dBm max). The value of τ_1 was adjusted to a ^{1}H suppression tau near a maximum in the ^{2}H nuclear modulation envelope (ca. 412 ns), and T was varied between -60 ns and a τ_2 setting for maximum detectable modulation periods of the expected dipolar frequency, in increments of 20 or 28 ns. The pump frequency ν_2 was centered in the resonator mode and aligned with the spectral maximum. The probe frequency ν_1 was 65 MHz above ν_2. When signal to noise was less than desirable with τ_2 = 7 μs, an additional trace was acquired with τ_2 = 5 μs. Data were analyzed and fit with DeerAnalysis2011 [28].

Determination of camphor dissociation rate

The following method is based on the approach by Griffin and Peterson [1]. Stopped-flow measurements were carried out on an Olis RSM apparatus. In syringe A, 5 μM P450cam was placed in 50 mM Tris (pH 7.4), 0-300 mM KCl and 0.2 mM camphor. In addition, 0, 1 and 2 equivalent of Pdx was included in syringe A. In syringe B, 5 mM metyrapone was placed in 50 mM Tris (pH 7.4) and 0-300 mM KCl. After rapid mixing of camphor-bound P450cam and metyrapone, UV-visible spectra were obtained every 1 ms for 1 sec. The kinetic data were fit to a single exponential model for the transition from the camphor-bound P450cam to the metyrapone-bound P450cam.

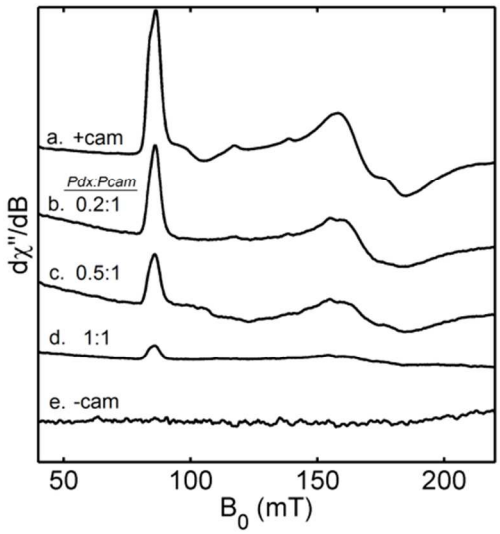

Figure S1. X-band EPR of aerobic P450 titrated with Pdx, measured at 15K.

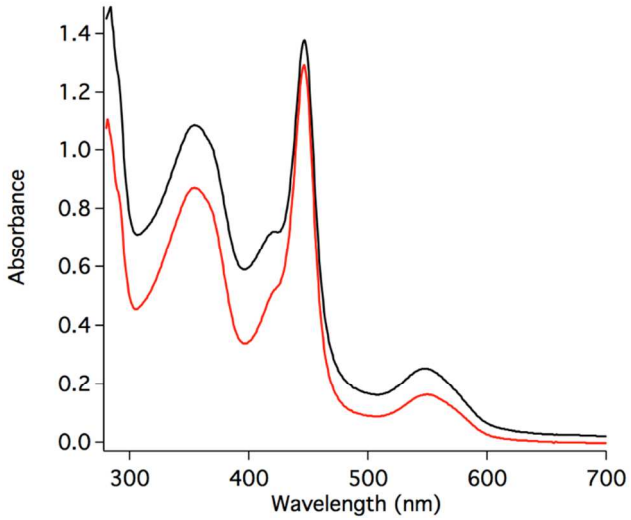

Figure S2. UV-vis spectra of CO-bound ferrous P450cam DEER samples. P450cam:Pdx = 1: 0.02 (red) and 1:1 (black).

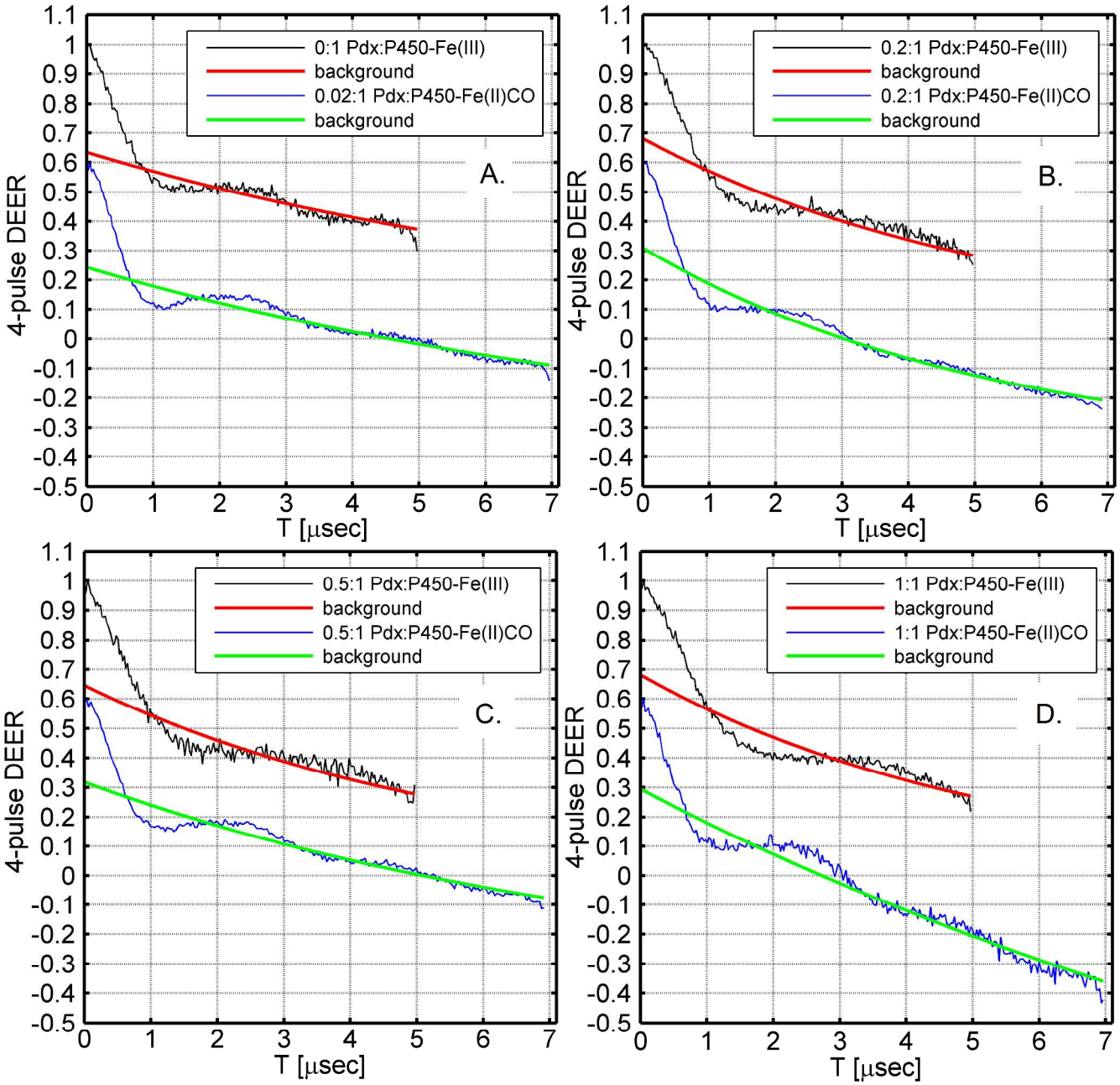

Figure S3. Raw DEER data and the background subtraction line used for generation of Figure 1. in the main text. The slight difference in modulation depth for the Fe(II)CO trace in panel C. is due to a pump pulse length of 20 ns vs. 16 ns used in all other acquisitions. The onset of pump/observe overlap is seen at the end of the P450-Fe(III) trace in panel A.